

pNF κ B-DD-ZsGreen1 Reporter Vector Map

Description

pNF κ B-DD-ZsGreen1 is a reporter vector that allows you to monitor NF κ B activation in mammalian cells. The vector contains an NF κ B enhancer element (composed of four tandem copies of the NF κ B consensus binding sequence; 1) upstream of a minimal TA promoter (P_{TA}), which consists of the TATA box from the herpes simplex virus thymidine kinase (HSV-TK) promoter. The vector encodes the reporter protein DD-ZsGreen1, a ligand-dependent, destabilized green fluorescent protein that minimizes background fluorescence from leaky promoters.

ZsGreen1 is a human codon-optimized variant of the reef coral *Zoanthus sp.* green fluorescent protein (ZsGreen) that has been engineered for brighter fluorescence (excitation and emission maxima: 493 and 505 nm, respectively; 1, 2). DD-ZsGreen1 is a modified version of ZsGreen1 that is tagged on its N-terminus with the ProteoTuner™ destabilization domain (DD; 3). The presence of this destabilization domain causes rapid, proteasomal degradation of the fluorescent fusion protein; however, when the membrane permeant ligand Shield1 is added to the medium, it binds to the destabilization domain and protects the fusion protein from degradation.

In the absence of Shield1, the destabilization domain causes the degradation of any DD-ZsGreen1 reporter protein produced prior to promoter activation, thus reducing background fluorescence. In order to analyze NF κ B activation, an inducer of choice is added to the medium along with the Shield1 stabilizing ligand, which effectively stabilizes the reporter protein, allowing it to accumulate. As a result, only the reporter molecules expressed during promoter induction will contribute to the fluorescence signal, providing a considerably higher signal-to-noise ratio than that obtained with non-destabilized or constitutively destabilized reporter systems. The high signal-to-noise ratio also allows the monitoring of NF κ B activation during discrete windows of time when Shield1 is added to the cell medium for discrete periods of time.

(PR9Z3417; published 8 January 2010)



Clontech

United States/Canada
800.662.2566

Asia Pacific
+1.650.919.7300

Europe
+33.(0)1.3904.6880

Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A neomycin-resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418 (4). This cassette consists of the SV40 early promoter, aTn5 kanamycin/neomycin resistance gene, and herpes simplex virus thymidine kinase (HSVTK) polyadenylation signals. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

Use

The pNF κ B-DD-ZsGreen1 Reporter vector, available as part of the NF κ B DD Green Reporter System (Cat. No. 631079), can be used to monitor NF κ B activation in live cells as well as *in vivo*. pNF κ B-DD-ZsGreen1 Reporter can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418.

Location of features

- NF κ B enhancer element: 54–93
- P_{TA} (TA minimal promoter): 113–119
- Kozak sequence: 214–224
- DD-ZsGreen1
 - Start codon (ATG): 221–223; Stop codon: 1244–1246
 - DD (FKBP-L106P destabilization domain; 3): 221–544
 - ZsGreen1 (*Zoanthus sp.* green fluorescent protein): 551–1243
- SV40 early polyA signals: 1399–1433
- f1 origin of replication: 1496–1951 (complementary)
- SV40 origin of replication: 2292–2430
- Kan^r/Neo^r (kanamycin/neomycin resistance gene)
 - Neomycin phosphotransferase coding sequences: 2476–3270
- HSVTK polyA signals: 3506–3524
- pUC origin of replication: 3855–4498

Propagation in *E. coli*

- Recommended host strains: DH5 α TM, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 μ g/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high
- Plasmid incompatibility group: pMB1/ColE1

Excitation and emission maxima of ZsGreen1

- Excitation maximum = 493 nm
- Emission maximum = 505 nm

References

1. Pessara, U. & Koch, N. (1990) *Mol. Cell Biol.* **10**(8):4146–4154.
2. Matz, M. V. *et al.* (1999) *Nature Biotech.* **17**(10):969–973.
3. Haas, J. *et al.* (1996) *Curr. Biol.* **6**(3):315–324
4. Banaszynski, L. *et al.* (2006) *Cell* **126**(5):995–1004.
5. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II*. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143–190.

Note: The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

Notice to Purchaser

Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of Clontech Laboratories, Inc.

DH5 α [™] is a trademark of Invitrogen Corporation.

Living Colors Fluorescent Protein Products:

Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or Clontech Customer Service at 650.919.7300. At its discretion, Clontech grants Not-For-Profit Entities a non-exclusive, personal, limited license to use this product for non-commercial life science research use only. Such license specifically excludes the right to sell or otherwise transfer this product, its components or derivatives thereof to third parties. No modifications to the protein coding sequence may be made without express written permission from Clontech. Any other use of this product requires a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com.

For-Profit Entities wishing to use this product are required to obtain a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com or [click here](#) for more information.

ProteoTuner[™] Protein Stabilization/Destabilization Products:**Reef Coral Fluorescent Proteins (RCFPs):**

The RCFP's (including DsRedExpress and DsRedExpress2) are covered by one or more of the following U.S. Patent Nos. 7,166,444; 7,157,565; 7,217,789; 7,338,784; 7,338,783; 7,537,915 6,969,597, 7,150,979 and 7,442,522.

Clontech, the Clontech logo and all other trademarks are the property of Clontech Laboratories, Inc., unless noted otherwise. Clontech is a Takara Bio Company. ©2010 Clontech Laboratories, Inc.